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Original article

Grass pollen symptoms interfere with the recollection of birch pollen symptoms – a prospective study of suspected, asymptomatic skin sensitization

Background: Asymptomatic skin sensitization (AS) is a risk factor for the development of allergic symptoms. A meticulous definition of this condition requires a systematic assessment of clinical symptoms before inclusion.
Objective: To examine the concordance between retrospective assessment of seasonal allergic symptoms and prospective seasonal symptom registration among subjects with AS.
Methods: On the basis of a population survey, autumn 2002, including skin prick tests (positive if ≥3 mm) and a screening questionnaire, 87 subjects with AS to birch and/or grass pollen, birch and/or grass pollen allergic symptomatic subjects ($n = 63$) and healthy controls ($n = 40$) were included in January to March 2003, completed diary cards on symptom and medication use during the relevant seasons 2003, and were examined at follow up in autumn 2003. Allergy: positive SPT and symptoms ≥ seven diary days.
Results: Eleven AS subjects (birch: $n = 10$) subsequently developed allergic symptoms, yet nine admitted, at follow up, to have had symptoms before inclusion, or even denied pollen-related symptoms despite a significant diary. Compared with AS subjects sensitized to grass pollen, AS subjects sensitized to birch pollen had significantly larger skin prick reactions and more often and severe pollen symptoms.
Conclusion: In the context of double-sensitization, retrospective symptom assessment is not a reliable method for ensuring that subjects classified, as asymptomatically skin sensitized, are truly, asymptomatic. This matter should be considered in studies on allergy development.

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Key words: allergy; asymptomatic skin sensitization; birch pollen; conversion rate; grass pollen; recollection; retrospective; symptom diary.

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Immunoglobulin E (IgE) skin sensitization and concomitant symptoms often coexist, but asymptomatic skin sensitization (AS) was described simultaneously with the introduction of routine skin testing (1). The prevalence of AS has been variably reported between 7.5% and 29.6%, most likely due to differences in age groups, number of allergens included, and definitions of skin test positivity (1–4). Both children and adults with AS have an increased risk of developing allergic symptoms compared with non-sensitized subjects, with annual conversion rates from 1.9% to 20.0% (1, 2). It has never been elucidated whether subjects with out-seasonally reported AS actually are symptom-free during relevant allergen exposure, except for a single study with a small cohort of students, where 60% of subjects with birch pollen AS developed allergic symptoms during 3 years of follow up (2). The finding that more subjects report to be pollen allergic when asked during the season than during the winter, suggests that out-seasonal underreporting of symptoms is a possibility (5).

This study aimed at investigating the consistency between an initial negative clinical history of hay fever to birch or/and grass pollen, among subjects with AS to birch or/and grass pollen, and their subsequent diary card symptom registration.

Abbreviations: AS, Asymptomatic skin sensitization; HC, healthy controls; NPV, negative predictive value; PPV, positive predictive value; SAR, seasonal allergic rhinitis; SPT, skin prick test; SQ-*U*, standardized quality-units.

Materials and methods

Participants

Using a mobile SPT unit at Universities in Copenhagen Denmark, in autumn 2002 (postseason), we screened subjects for either AS or seasonal allergic rhinitis (SAR) by skin prick tests [SPT positive if ≥ 3 mm (6)], and a screening questionnaire (questions listed in

Table 1. Association between self-reported pollen related symptoms and skin prick test positivity in the screening survey 2002

'Have you within this year or in previous years experienced..	Sensitivity (%)*	Specificity (%)*	Odds ratio (95% CI)†
1a) itchy/running nose and/or red/itchy/running eyes when near trees primarily in April/May?' *vs* SPT birch	44.7	91.5	9.1 (7.0–12.0)
1b) wheezing and/or strenuous breathing and/or dry cough when near trees primarily in April/May?' *vs* SPT birch	7.5	99.0	7.0 (3.9–12.5)
1a) or 1b) *vs* SPT birch	45.0	91.3	9.5 (7.2–12.5)
2a) itchy/running nose and/or red/itchy/running eyes when near grass primarily in June/July?' *vs* SPT grass	63.6	89.0	14.0 (11.2–17.5)
2b) wheezing and/or strenuous breathing and/or dry cough when near grass primarily in June/July?' *vs* SPT grass	13.5	98.3	8.2 (5.3–12.6)
2a) or 2b) *vs* SPT grass	63.8	88.7	13.6 (10.8–17.0)

SPT, skin prick test.
$n = 2237$ (subjects with complete data on birch and grass pollen related symptoms).
*Sensitivity and specificity calculated with SPT as 'true diagnosis'.
†Odds ratio (adjusted for sex and age) for a positive SPT, presumed affirmative answer to 1a) or 1b); birch pollen; 2a) or 2b); grass pollen.

Table 1). The ISAAC protocol did not meet our requirements; instead we used a validated questionnaire by Linneberg *et al.* (7), shortened to our needs. The first screening questionnaire was completed during the 15 min preceding the registration of potential skin reactions. All participants gave written informed consent. The local Ethical Committee of Copenhagen approved the study.

Inclusion (January to March 2003)

Figure 1 depicts the inclusion procedure. After completing the screening study, we invited a selected cohort for inclusion: totally 167 participants were included (women: 56.7%, median age: 24.0 years; range: 18.6–63.6 years), and underwent SPT re-testing and a clinical interview. Figure 1 depicts the identification of three groups: (AS) group ($n = 87$); (SAR) group ($n = 63$) and a healthy controls (HC) group consisting of 40 subjects with a negative clinical history of any atopic symptoms, and a negative SPT to all 10 aeroallergens (see 'Skin tests'). It was observed that that 23 subjects had positive SPTs to both birch and grass pollen but were asymptomatic to one allergen source and symptomatic to the other (Fig. 1).

Due to logistic problems, merely 74.9% ($n = 125$) of the participants re-completed the same screening questionnaire at inclusion (prescason), the percentage of re-completed screening questionnaires being fairly equal among: AS: 78.0%, SAR: 76.2% and HC: 67.5%. This time, the screening questionnaire was completed after the clinical testing.

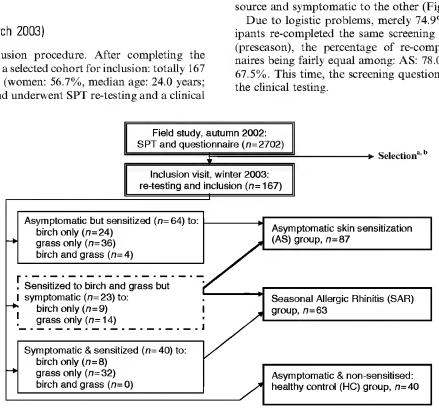

Figure 1. In January to March 2003, 167 persons were included. The included subjects contributed to the: AS group $n = 87$subjects, SAR group $n = 63$ subjects and HC group $n = 40$ healthy skin prick test negative subjects. Twenty-three subjects had positive SPTs to both birch and grass pollen but were asymptomatic to one allergen source and symptomatic to the other (marked with a dashed box and thicker black arrows).

At the time of the postseasonal screening questionnaire, the positive predictive value (PPV) of an affirmative answer (questions listed in Table 1), for a similar affirmative answer at the time of inclusion was 97.6%. Similarly, the negative predictive value (NPV) of an initial negative answer, for a subsequent similar negative answer was 92.8%. In case of discrepancies between the postseasonal and preseasonal screening questionnaire, the final clinical status of the participant was settled after the clinical interview.

Exclusion criteria were pregnancy or breast-feeding, perennial rhinitis or asthma, continuous treatment with anti-histaminic drugs, severe chronic disease, and expected low compliance. In AS and SAR groups, multiple SPT sensitization was allowed.

Skin tests

The standard SPT used for the screening survey comprised *Betula verrucosa*, *Phleum pratense*, *Artemisia vulgaris*, horse, dog, cat dander, *Dermatophagoides pteronyssinus*, *D. farinae*, *Cladosporium herbarum*and *Alternaria alternata* (100 000 SQ-*U*; Soluprick, ALK-Abello Hørsholm, Denmark). At inclusion, we performed a titrated SPT with 100 000, 33 000, and 10 000 SQ-*U* birch and/or grass pollen extract. All skin tests were performed on the volar surface of the antebrachium (in duplicate), with histamine dihydrochloride (10 mg/ml) as positive and diluent as negative controls (6).

Pollen counts and seasons

Pollen grains were collected by a standard Burkard Volumetric 7-days spore trap in Copenhagen, Denmark, 15 m above ground level. Pollen grains were counted daily (numbers/m^3) by the Danish Meteorological Institute. The birch and grass pollen seasons overlap (8), and birch-related symptoms persist weeks after the decline in birch pollen counts (5). Thus, we defined the 'birch pollen season' as the period starting the first day with birch pollen levels >10 grains/m^3 and ending, when grass pollen reached a level of 10 grains/m^3.

In-seasonal symptom and medication registration

Daily diary cards on symptoms and medication were completed: during the pollen season(s) 2003 corresponding to sensitization (AS and SAR groups), or both (HC). All cards were collected and symptoms from nose, eyes and lungs were graded from 0 to 3 (0, none; 1, mild; 2, moderate; 3, severe symptoms). The 'symptom score' was calculated as the sum of daily organ related scores during the season. Symptoms were considered as pollen-related when lasting ≥ 7days (2), and termed 'allergy' when accompanied by relevant SPT sensitization. All participants had free access to standard rescue medication, which was scored as tablet loratadine 10 mg (two points), each drop of levocabastine 0.5 mg/ml or nasal spray 50 μg/dose (both one point). If symptoms were not controllable on maximal antihistamine drug treatment, local and systemic steroids were accepted: one puff of nasal steroid 50 μg/dose (one point), oral prednisolone (12.5 mg/day) (two points), and injection of methylprednisolone 40 mg/ml [estimated as corresponding to 21 days of oral prednisolone (12.5 mg/day)] and thus 42 points (9). The individual symptom and medication score were summed to a 'total symptom load score'.

Postseasonal symptom and medication registration (follow up)

All participants were interviewed in autumn 2003 with regard to seasonal airway symptoms, as at the inclusion visit (questions in Table 1). A titrated SPT was performed.

Statistical analysis

Using SPSS version 11.1 (Chicago, IL, USA), differences between groups were analysed using the Mann–Whitney *U*-test (continuous variables) and Chi-square-test/Fisher's exact test (binary variables). Subjects not attending follow up or without completed diaries were excluded from further analyses. Statistical significance was considered at $P < 0.05$.

Results

Prevalence of SPT positivity

In the screening population, complete data were available in 2237 subjects (58.6% women, median age 24.1; range 18.0–75.3 years). The diagnostic rates of the screening questionnaire (Table 1) of rhino-conjunctival symptoms to grass pollen in relation to SPT positivity to grass pollen (Table 1), matched those by Linneberg *et al.* (7), whereas the sensitivity of birch-related hay-fever symptoms was lower.

Season and exclusion

The 'birch pollen season' lasted from the 21 April to the 3 June 2003, covering >99% of the total birch pollen exposure (10), the 'grass pollen season' from the 3 June to the 31 July 2003, covering >99% of the total grass pollen exposure (10). Both the birch and the grass pollen season 2003 were relatively mild (2). All subjects were living in Copenhagen during birch pollinosis. All subjects in the HC, AS and SAR groups were anamnestically (e.g. mowed the lawn) and geographically exposed to *P. pratense* during grass pollinosis.

Nine AS subjects were excluded due to missing diaries (AS birch pollen: $n = 4$; AS grass pollen $n = 5$), leaving nAS = 78. Seven SAR subjects were excluded from further analysis mainly as dropouts (SAR birch pollen: $n = 1$; SAR grass pollen $n = 6$), leaving nSAR = 56. None in the HC group was excluded.

Agreement between out- and in-seasonal symptoms registration

In the AS group, 11 participants (11/78 = 14.1%; AS birch: $n = 10$) reported allergic symptoms ≥7 days during the relevant season, and thus had 'allergy' (Table 2). At inclusion, eight of the 10 subjects with AS to birch pollen reported grass pollen hay fever, which remained their main problem [total symptom load score', median (range): birch pollen: 39.5 (8.0–154.0), grass pollen: 145.5 (12.0–203.0), $P < 0.05$, Mann–Whitney *U*-test]. At the follow-up visit, seven out of these eight admitted to have had birch pollen related symptoms before inclusion, or continued to deny having had such symptoms during 2003. Of the remaining three subjects, one recognized to have had symptoms before inclusion. Thus, only two of 78 AS subjects (2.6%) were reliable converters from AS

Table 2. Differences between asymptomatic subjects with or without allergy to other allergen source

	Asymptomatic ($n = 64$*)	Symptomatic to other allergen source ($n = 23$†)	*P*-value
Preseasonal			
SPT at inclusion in mm, median (range)	4.8 (3.0–12.5)	6.5 (3.0–14.5)	$P < 0.01$**
In-seasonal			
Total symptom load score, median (range)	0.0 (0.0–288.0)	6.0 (0.0–154.0)	$P < 0.001$**
Medication use (*n* cases/*n* total)	1/58[a]	7/20[b]	$P < 0.001$††
Allergy (*n*cases/*n*total)	3/58[a]	8/20[b]	$P = 0.001$††

SPT, skin prick test.

All variables listed refers to the allergen to which subjects were asymptomatically sensitized at inclusion.

*Sensitized to birch ($n = 24$), grass ($n = 36$) or both ($n = 4$).

†Sensitized to both birch and grass, but symptoms to birch ($n = 9$) or grass ($n = 14$).

[a,b]Information on nine subjects with AS was lost at follow up, thus of the remaining (64 + 23) − 9 = 78 individuals with AS, 58 subjects were purely asymptomatic (a) while 20 subjects (b) were symptomatic to another allergen source.

**Mann–Whitney *U*-test; ††Fischer's exact test.

to SAR. Table 2 shows that AS subjects with and without other allergy differed significantly in both paraclinical and clinical aspects.

In the SAR group, two subjects (3.6%) reported no symptoms and had negative diaries at follow up. Five subjects with SAR (8.9%) reported symptoms, but had diary confirmed symptoms <7 days. All subjects in the AS and SAR group had at least two positive SPTs; however, two subjects with AS (both remaining asymptomatic, 2.6%) and one subject with SAR (symptoms ≥7 days, 1/56 = 1.8%) failed to reproduce positive SPTs at follow up. None in the HC group admitted symptoms at follow up, but seven (17.5%) HC had diary confirmed symptoms; however, none ≥7 days, and none developed new sensitizations at follow up.

Discussion

Theoretically, respiratory symptoms to a particular allergen may be misinterpreted as common cold, airway irritants, or allergy to other allergens (11). Furthermore, the presence of clinically significant allergies may overshadow the recollection of symptoms from clinically less dominant allergens, similar to the context dependence of pain recollection (12). In addition, temporal proximity between pollen seasons may also lead to confusion with regard to retrospective symptom registration.

We included university students, to investigate the agreement between retrospective symptom assessment and prospective symptom registration among subjects with AS, using allergic symptoms (symptoms ≥7 days) as outcome variable (2). Major selection bias with respect to IgE skin sensitization was unlikely, as the frequency of SPT positivity, respectively the distribution of SPT positivity to allergen subgroups, in our screening population, matched those found in randomized Scandinavian studies (13, 14) (data not shown). SPT reproducibility in both the AS and SAR groups was high. The included HC group functioned as a control for unspecific airway symptoms (15), while the included subjects with SAR were used to substantiate our choice of clinical symptom threshold. Total suppression of symptoms due to medication was not observed in our study, as only AS and SAR subjects reporting allergic symptoms used antiallergic medication. This observation also substantiated the clinical relevance of our symptom threshold.

We found a conversion rate from AS to SAR of 14.1% (11/78 subjects), but only 2.6% (2/78) were true converters as the remaining subjects recognized to have had symptoms before inclusion or persisted to deny symptoms out-seasonally despite significant in-seasonal symptom registration. We found that the presence of another, more pronounced respiratory allergy negatively influenced the validity of out-seasonally recorded symptoms. This is consistent with retrospective overestimation of symptoms among birch pollen allergies (16) as well as the better concordance in this study between out-seasonal and in-seasonal symptom status observed in the SAR group compared with the AS group. We included three symptom recordings: post-, pre-, and in-seasonal within 12 months. The short time span between inclusion and follow up, covering relatively mild pollen seasons, makes it plausible that initial symptom misclassification rather than a natural history of allergy development accounts for our findings. Differences in pollen exposure due to urban *vs* rural location do not seem to account for our results, as all subjects were living in the capital (Copenhagen) during birch pollen exposure, and all subjects in the HC, AS and SAR groups were exposed to *P. pratense* during grass pollinosis.

In a prospective study by Horak, AS participants were questioned about allergic airway symptoms before and after the season (4). The conversion rate in the first season was >30%, but 5–10% in the following three seasons without much fluctuation in annual pollen load (Dr Friedrich Horak, personal communication). This indicates that information obtained when entering a clinical study *per se* increases symptom awareness.

Remes *et al.* found that the prevalence of birch pollen related symptoms was lower when data were obtained during winter than spring (5). Our screening questionnaire contained detailed information on the periods of birch and grass pollination. The first screening questionnaire was completed in October and November 2002, in rather close approximation to the grass pollen season. This could explain the better agreement between post- and in-seasonal reporting of grass pollen symptoms than birch pollen symptoms. However, we found that our screening questionnaire was reproducible when completed during late winter (inclusion, January to March 2003), substantiating that other factors, than merely the time span between pollination and symptoms registration, were implicated in the observed memory bias concerning birch pollen related symptoms (Table 1). Though the second screening questionnaire (inclusion 2003) was completed after the clinical testing, the high degree of correspondence with the results obtained from the first screening questionnaire (survey 2002), indicates that the completion of questionnaires in general, was unaffected by recently acquired knowledge of SPT results.

In conclusion, our results emphasize the complexity of symptom registration: by a thorough exploration of the clinical history, the annual conversion rate was reduced from 14.1% to 2.6%. In this, the largest study on AS, using diaries, our data do not support our earlier findings in birch pollen AS (annual conversion rates of allergy of 20%), in a small-scale study also relying on symptom diaries (2).

We confirmed that a restrospectively obtained history is reliable when supported by a matching SPT result, and that the symptoms diary is the golden standard of symptom registration in clinical allergology: when SPT and history do not match, conversion rates of AS should be calculated after thorough exploration of the history and with the first symptom diary as baseline.

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